Another object of the invention is to provide a biotechnological process for reacting carboxylic acid esters to give aminated carboxylic acid esters, which is as efficient as possible with regard to yield, carbon balance and/or nitrogen balance, re-usability of agents used and/or purity of the product. In this connection, an efficient carbon balance and/or nitrogen balance preferably means that the proportion of the carbon and/or nitrogen fed in the form of suitable substrates to a cell for reacting a carboxylic acid ester is as high as possible in the desired final product, instead of being reacted to give products other than the desired ones, for example.

Another object of the invention is to improve the ability of a multi-phase reaction mixture to be worked up from the reaction of a carboxylic acid ester, particularly with regard to re-usability of hydrophobic solvents and liquid cation exchangers used for work-up, and with regard to phase formation and separation in a biphasic system comprising an aqueous phase in which reaction of the carboxylic acid ester takes place and an organic phase with organic solvents and/or liquid cation exchangers.

These and further objects are achieved by the subject matter of the present application and in particular also by the subject matter of the enclosed independent claims, with the dependent claims giving rise to embodiments.

In one example, the problem addressed by the disclosure is solved by a process for reacting a carboxylic acid ester of the formula (I)

$$R^1 - A - COOR^2 \quad (I),$$

wherein R^1 is selected from the group comprising hydrogen, $-CH_2OH$, $-CHO$, $-COOR^3$, $-CH_2SH$, $-CH_2OR^3$ and $-CH_2NH_2$,

wherein R^2 is selected from the group comprising hydrogen and alkyl, preferably methyl, ethyl and propyl,

wherein R^3 is selected from the group comprising hydrogen and alkyl, preferably methyl, ethyl and propyl,

and wherein A is a substituted, unsubstituted, linear, branched and/or cyclic alkylene, alkenylene, arylene or aralkylene radical having at least 4, more preferably 6, even more preferably 8, carbons,

by means of a cell, comprising the step of

a) contacting the cell with said carboxylic acid ester in an aqueous solution,

wherein the cell is a recombinant cell which has reduced activity of a polypeptide comprising SEQ ID NO 2 or a variant thereof over the wild-type cell.

In another example, the problem is solved by a process further comprising the step of

b) contacting the aqueous solution with a hydrophobic organic solution comprising a cation exchanger.

In another example, the problem addressed by the disclosure is solved by use of a knockout of a gene coding for a polypeptide comprising SEQ ID NO 2 or a variant thereof as part of the genetic make-up of a recombinant cell for increasing production of a carboxylic acid ester of the formula (I)

$R^1 - A - COOR^2$ (I),

wherein R^1 is selected from the group comprising hydrogen, $-CH_2OH$, $-CHO$, $-COOR^3$, $-CH_2SH$, $-CH_2OR^3$ and $-CH_2NH_2$,

wherein R^2 is selected from the group comprising alkyl, preferably methyl, ethyl and propyl,

wherein R^3 is selected from the group comprising hydrogen and alkyl, preferably methyl, ethyl and propyl,

and wherein A is a substituted, unsubstituted, linear, branched and/or cyclic alkylene, alkenylene, arylene or aralkylene radical having at least 4, more preferably 6, even more preferably 8, carbons, in particular an alkylene radical having at least four, more preferably 6, even more preferably 8, carbons,

over the corresponding wild-type cell.

In another example, the problem addressed by the disclosure is solved by using a recombinant cell which has reduced activity of a polypeptide having SEQ ID NO 2 or a variant thereof over the wild-type cell for reacting a carboxylic acid ester of the formula (I)

$R^1 - A - COOR^2$ (I),

wherein R^1 is selected from the group comprising hydrogen, $-CH_2OH$, $-CHO$, $-COOR^3$, $-CH_2SH$, $-CH_2OR^3$ and $-CH_2NH_2$,

wherein R^2 is selected from the group comprising hydrogen and alkyl, preferably methyl, ethyl and propyl,

wherein R^3 is selected from the group comprising hydrogen and alkyl, preferably methyl, ethyl and propyl,

and wherein A is a substituted, unsubstituted, linear, branched and/or cyclic alkylene, alkenylene, arylene or aralkylene radical having at least 4, more preferably 6, even more preferably 8, carbons, preferably an alkylene radical having at least eight carbons.

In another example, the problem is solved by a process or a use, wherein A is a saturated alkylene radical, preferably an alkylene radical of the formula $-(CH_2)_n-$, where n is at least 4.

In another example, the problem is solved by a process, wherein R^1 is selected from the group comprising hydrogen, $-CH_2OH$, $-CHO$ and $-CH_2NH_2$.

In another example, the problem addressed by the disclosure is solved by a cell expressing a recombinant alkane hydroxylase, wherein the activity of a polypeptide comprising SEQ ID NO 2 or a variant thereof is reduced over the wild-type cell.

In a preferred example, the problem is solved by a cell, wherein the alkane hydroxylase is an alkane hydroxylase from the group comprising AlkB monooxygenases and a cytochrome P450 monooxygenase of the CYP153 family.

In one aspect, the present disclosure refers to a process for transforming a carboxylic acid ester of the formula (I) into a second carboxylic acid ester:

$$R^1 - A - COOR^2 \quad (I),$$

where

R^1 is hydrogen, $-CH_2OH$, $-CHO$, $-COOR^3$, $-CH_2SH$, $-CH_2OR^3$ and $-CH_2NH_2$,

R^2 is an alkyl group,

R^3 is hydrogen or an alkyl group, and

A is an unsubstituted, a linear, or an unsubstituted and linear alkylene radical having at least 10 carbons,

the process comprising:

contacting a recombinant cell with the carboxylic acid ester of the formula (I) in an aqueous solution to convert the carboxylic acid ester of the formula (I) to the second carboxylic acid ester,

wherein the recombinant cell has a reduced activity of a polypeptide comprising SEQ ID NO: 2 or a polypeptide having at least 70% homology to SEQ ID NO: 2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO: 2 compared to an activity in a corresponding wild-type cell due to knock-out of a gene encoding the polypeptide comprising SEQ ID NO: 2 or the polypeptide having at least 70% homology to SEQ ID NO: 2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO: 2,

the recombinant cell comprises a polypeptide having at least 80% sequence identity to consecutive 230 amino acids of SEQ ID NO: 15 and having essentially the same enzymatic activity as *Pseudomonas putida* AlkL, where the polypeptide is optionally overexpressed,

the recombinant cell has been transferred to express a transaminase, *Pseudomonas putida* AlkBGT, and an alanine dehydrogenase,

the recombinant cell has a reduced activity of at least one enzyme catalyzing any of reactions of fatty acid β-oxidation compared to an activity in the wild-type cell due to knock-out, and

the at least one enzyme catalyzing any of reactions of fatty acid β-oxidation is selected from the group consisting of fatty acid-CoA ligase, acyl-CoA

dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase, 3-ketoacyl-CoA thiolase, and a fatty acid importer.

In one example, the problem addressed by the disclosure is solved by a reaction mixture comprising the cell according to the examples in aqueous solution and a carboxylic acid ester of the formula (I)

$$R^1 - A - COOR^2 \quad (I),$$

wherein R^1 is selected from the group comprising hydrogen, $-CH_2OH$, $-CHO$, $-COOR^3$, $-CH_2SH$, $-CH_2OR^3$ and $-CH_2NH_2$,

wherein R^2 is selected from the group comprising alkyl, preferably methyl, ethyl and propyl,

wherein R^3 is selected from the group comprising hydrogen and alkyl, preferably methyl, ethyl and propyl,

wherein A is a substituted, unsubstituted, linear, branched and/or cyclic alkylene, alkenylene, arylene or aralkylene radical having at least 4, more preferably 6, even more preferably 8, carbons.

In another example, the problem is solved by the reaction mixture,

wherein A is an alkylene radical having at least four carbons, in particular a saturated alkylene radical, preferably an alkylene radical of the formula $-(CH_2)_n-$, where n is at least 4,

wherein R^1 is preferably selected from the group comprising hydrogen, $-CH_2OH$, $-CHO$ and $-CH_2NH_2$.

In another example, the problem is solved by a reaction mixture further comprising a hydrophobic organic solution containing a cation exchanger.

In a preferred example, the problem is solved by a process, a cell or a use, wherein the cell furthermore expresses a transaminase.

In another preferred example, the problem is solved by a process, a cell or a use, wherein the cell furthermore expresses an alanine dehydrogenase.

In another preferred example, the problem is solved by a process, a cell or a use, wherein the cell furthermore has a protein of the AlkL family.

In another preferred example, the problem is solved by a process, a cell or a use, wherein the cell has an activity, which is reduced over its wild type, of at least one enzyme catalysing any of the reactions of fatty acid β-oxidation, wherein the enzyme is preferably one from the group comprising fatty acid-CoA ligase, acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase, a fatty acid importer or variants thereof, particularly preferably FadL or a variant thereof.

In another preferred example, the problem is solved by a process, a cell or a use, wherein the cell has and/or overexpresses at least one enzyme from the group comprising alkane hydroxylase, alcohol dehydrogenase, transaminase, alanine dehydrogenase and protein of the AlkL family in recombinant form.

In another preferred example, the problem is solved by a process, a cell or a use, wherein the activity of a polypeptide comprising SEQ ID NO 2 or a variant thereof is reduced over the wild-type cell due to knockout of a gene coding for a polypeptide comprising SEQ ID NO 2 or a variant thereof.

In another aspect, the present disclosure refers to a method for producing a carboxylic acid ester of the formula (I') from a substrate carboxylic acid ester:

$$R^1 - A - COOR^2 \qquad (I'),$$

where

R^1 is hydrogen, $-CH_2OH$, $-CHO$, $-COOR^3$, $-CH_2SH$, $-CH_2OR^3$ or $-CH_2NH_2$,

R^2 is an alkyl group,

R^3 is hydrogen or an alkyl group, and

A is an unsubstituted, a linear, or an unsubstituted and linear alkylene radical having at least 10 carbons,

the method comprising:

contacting a recombinant cell with the substrate carboxylic acid ester in an aqueous solution to convert the substrate carboxylic acid ester to the carboxylic acid ester of the formula (I'),

wherein the recombinant cell comprises a knockout of a gene encoding a polypeptide comprising SEQ ID NO: 2 or a polypeptide having at least 70% homology to SEQ ID NO:2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO:2,

the recombinant cell comprises a polypeptide having at least 80% sequence identity to consecutive 230 amino acids of SEQ ID NO: 15 and having essentially the same enzymatic activity as *Pseudomonas putida* AlkL, where the polypeptide is optionally overexpressed,

the recombinant cell has been transformed to express a transaminase, *Pseudomonas putida* AlkBGT, and an alanine dehydrogenase,

the production of the carboxylic acid ester of the formula (I') is increased over a corresponding wild-type cell of the recombinant cell,

the recombinant cell has a reduced activity of at least one enzyme catalyzing any of reactions of fatty acid β-oxidation compared to an activity in the wild-type cell due to knockout, and

the at least one enzyme catalyzing any of reactions of fatty acid β-oxidation is selected from the group consisting of fatty acid-CoA ligase, acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase, 3-ketoacyl-CoA thiolase, and a fatty acid importer.

The invention is based on the inventors surprisingly finding that a recombinant cell which has reduced activity of a polypeptide comprising SEQ ID NO 2 in particular or a variant thereof over the wild-type cell is suitable for reacting carboxylic acid esters in that yield, carbon balance and/or nitrogen balance and purity of the products produced therefrom are surprisingly higher than with a cell that has the same activity as the wild-type cell with regard

Patent Claims

1. A process for transforming a carboxylic acid ester of the formula (I) into a second carboxylic acid ester:

$$R^1 - A - COOR^2 \quad (I),$$

where

R^1 is hydrogen, $-CH_2OH$, $-CHO$, $-COOR^3$, $-CH_2SH$, $-CH_2OR^3$ and $-CH_2NH_2$,

R^2 is an alkyl group,

R^3 is hydrogen or an alkyl group, and

A is an unsubstituted, a linear, or an unsubstituted and linear alkylene radical having at least 10 carbons,

the process comprising:

contacting a recombinant cell with the carboxylic acid ester of the formula (I) in an aqueous solution to convert the carboxylic acid ester of the formula (I) to the second carboxylic acid ester,

wherein the recombinant cell has a reduced activity of a polypeptide comprising SEQ ID NO: 2 or a polypeptide having at least 70% homology to SEQ ID NO: 2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO: 2 compared to an activity in a corresponding wild-type cell due to knock-out of a gene encoding the polypeptide comprising SEQ ID NO: 2 or the polypeptide having at least 70% homology to

SEQ ID NO: 2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO: 2,
the recombinant cell comprises a polypeptide having at least 80% sequence identity to consecutive 230 amino acids of SEQ ID NO: 15 and having essentially the same enzymatic activity as *Pseudomonas putida* AlkL, where the polypeptide is optionally overexpressed,
the recombinant cell has been transferred to express a transaminase, *Pseudomonas putida* AlkBGT, and an alanine dehydrogenase,
the recombinant cell has a reduced activity of at least one enzyme catalyzing any of reactions of fatty acid β-oxidation compared to an activity in the wild-type cell due to knock-out, and
the at least one enzyme catalyzing any of reactions of fatty acid β-oxidation is selected from the group consisting of fatty acid-CoA ligase, acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase, 3-ketoacyl-CoA thiolase, and a fatty acid importer.

2. The process according to Claim 1, further comprising:

contacting the aqueous solution with a hydrophobic organic solution comprising a cation exchanger.

3. The process of claim 1, wherein
R^2 is methyl, ethyl or propyl,
R^3 is methyl, ethyl or propyl, and
A is an unsubstituted, a linear, or an unsubstituted and linear alkylene radical having 11 carbons.

4. The process according claim 1, wherein A is an unsubstituted and linear alkylene radical.

5. The process according to claim 1, wherein R^1 is hydrogen, $-CH_2OH$, $-CHO$ and $-CH_2NH_2$.

6. The process according to claim 1, wherein A is an alkylene radical of formula $-(CH_2)_n-$, where n is at least 10.

7. The process according to claim 1, wherein the second carboxylic acid ester has the formula (I'):

$$R^1 - A - COOR^2 \quad (I'),$$

where

R^1 is hydrogen, $-CH_2OH$, $-CHO$, $-COOR^3$, $-CH_2SH$, $-CH_2OR^3$ and $-CH_2NH_2$,

R^2 is an alkyl group,

R^3 is hydrogen or an alkyl group, and

A is an unsubstituted, a linear, or an unsubstituted and linear alkylene radical having at least 10 carbons.

8. The process according to claim 1, wherein the carboxylic acid ester of the formula (I) is methyl dodecanoate.

9. The process according to claim 1, wherein the second carboxylic acid ester comprises methyl 12-amino-dodecanoate.

10. The process according to claim 1, wherein the at least one enzyme catalyzing any of reactions of fatty acid β-oxidation comprises at least one of FadA, FadB, FadD, FadL, and FadE from *Escherichia coli*.

11. The process according to claim 1,

wherein R^1 is hydrogen, $-CH_2OH$, $-CHO$ or $-CH_2NH_2$,

R^2 is methyl, ethyl or propyl, and

A is an alkylene radical of formula $-(CH_2)_n-$, where n is at least 10.

12. The process according to claim 1,

wherein the cell has a reduced activity of a polypeptide comprising SEQ ID NO: 2 or a polypeptide having at least

90% homology to SEQ ID NO:2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO:2 compared to an activity in a corresponding wild-type cell due to knockout of a gene encoding the polypeptide comprising SEQ ID NO:2 or the polypeptide having at least 90% homology to SEQ ID NO:2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO:2, and
the recombinant cell comprises a polypeptide having at least 90% sequence identity to consecutive 230 amino acids of SEQ ID NO: 15 and having essentially the same enzymatic activity as *Pseudomonas putida* AlkL.

13. The process according to claim 1,
wherein the cell has a reduced activity of a polypeptide comprising SEQ ID NO: 2 or a polypeptide having at least 96% homology to SEQ ID NO:2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO:2 compared to an activity in a corresponding wild-type cell due to knockout of a gene encoding the polypeptide comprising SEQ ID NO:2 or the polypeptide having at least 96% homology to SEQ ID NO:2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO:2, and
the recombinant cell comprises a polypeptide having at least 90% sequence identity to consecutive 230 amino acids of SEQ ID NO: 15 and having essentially the same enzymatic activity as *Pseudomonas putida* AlkL.

14. The process according to claim 1,
wherein the carboxylic acid ester of the formula (I) is methyl dodecanoate, and
the second carboxylic acid ester comprises methyl 12-amino-dodecanoate.

15. The process according to claim 14, wherein the recombinant cell is *Escherichia coli*, the transaminase

is *Chromobacterium violaceum* Cv_2025, and the alanine dehydrogenase is *Bacillus subtilis* ald.

16. The process according to claim 15, further comprising: after the contacting, extracting the second carboxylic acid ester from the aqueous solution by contacting the aqueous solution with a hydrophobic organic solution comprising a cation exchanger.

17. The process according to claim 16, wherein the recombinant cell comprises a polypeptide having the consecutive 230 amino acids of SEQ ID NO: 15.

18. A method for producing a carboxylic acid ester of the formula (I') from a substrate carboxylic acid ester:

$$R^1 - A - COOR^2 \qquad (I'),$$

where
R^1 is hydrogen, $-CH_2OH$, $-CHO$, $-COOR^3$, $-CH_2SH$, $-CH_2OR^3$ or $-CH_2NH_2$,
R^2 is an alkyl group,
R^3 is hydrogen or an alkyl group, and
A is an unsubstituted, a linear, or an unsubstituted and linear alkylene radical having at least 10 carbons,
the method comprising:
contacting a recombinant cell with the substrate carboxylic acid ester in an aqueous solution to convert the substrate carboxylic acid ester to the carboxylic acid ester of the formula (I'),
wherein the recombinant cell comprises a knockout of a gene encoding a polypeptide comprising SEQ ID NO: 2 or a polypeptide having at least 70% homology to SEQ ID NO:2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO:2,
the recombinant cell comprises a polypeptide having at least 80% sequence identity to consecutive 230 amino

acids of SEQ ID NO: 15 and having essentially the same enzymatic activity as *Pseudomonas putida* AlkL, where the polypeptide is optionally overexpressed,
the recombinant cell has been transformed to express a transaminase, *Pseudomonas putida* AlkBGT, and an alanine dehydrogenase,
the production of the carboxylic acid ester of the formula (I') is increased over a corresponding wild-type cell of the recombinant cell,
the recombinant cell has a reduced activity of at least one enzyme catalyzing any of reactions of fatty acid β-oxidation compared to an activity in the wild-type cell due to knockout, and
the at least one enzyme catalyzing any of reactions of fatty acid β-oxidation is selected from the group consisting of fatty acid-CoA ligase, acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase, 3-ketoacyl-CoA thiolase, and a fatty acid importer.

19. The method according to claim 18,
wherein the recombinant cell comprises a knockout of a gene encoding a polypeptide comprising SEQ ID NO: 2 or a polypeptide having at least 90% homology to SEQ ID NO:2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO:2, and
the recombinant cell comprises a polypeptide having at least 90% sequence identity to consecutive 230 amino acids of SEQ ID NO: 15 and having essentially the same enzymatic activity as *Pseudomonas putida* AlkL.

20. The method according to claim 18,
wherein the recombinant cell comprises a knockout of a gene encoding a polypeptide comprising SEQ ID NO: 2 or a polypeptide having at least 96% homology to SEQ ID NO:2 and having essentially the same enzymatic activity as the polypeptide comprising SEQ ID NO:2, and

the recombinant cell comprises a polypeptide having at least 90% sequence identity to consecutive 230 amino acids of SEQ ID NO: 15 and having essentially the same enzymatic activity as *Pseudomonas putida* AlkL.

5

Process for reacting a carboxylic acid ester

Abstract

The invention relates to a process for reacting a carboxylic acid ester of the formula (I)

$$R^1 - A - COOR^2 \quad (I),$$

wherein R^1 is selected from the group comprising hydrogen, $-CH_2OH$, $-CHO$, $-COOR^3$, $-CH_2SH$, $-CH_2OR^3$ and $-CH_2NH_2$,

wherein R^2 is selected from the group comprising alkyl, preferably methyl, ethyl and propyl,

wherein R^3 is selected from the group comprising hydrogen and alkyl, preferably methyl, ethyl and propyl,

and wherein A is a substituted, unsubstituted, linear, branched and/or cyclic alkylene, alkenylene, arylene or aralkylene radical having at least 4, more preferably 6, even more preferably 8, carbons,

by means of a cell, comprising the step of

a) contacting the cell with said carboxylic acid ester in an aqueous solution,

wherein the cell is a recombinant cell which has reduced activity of a polypeptide comprising SEQ ID NO 2 or a variant thereof over the wild-type cell, and to a cell suitable therefor, and to uses thereof.

(No suitable figure)

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